Running head: Salmonella and Campylobacter contamination of poultry

Full title:
Survey of Salmonella and Campylobacter contamination of whole, raw poultry on retail sale in Wales in 2003

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Short title:
Salmonella and Campylobacter in raw poultry

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Abstract

A survey of the *Salmonella* and *Campylobacter* contamination of raw, whole chickens available to consumers in Wales was carried out between March and December 2003. In total, 736 samples were taken and overall contamination rates of 73.1% for *Campylobacter* and 5.7% for *Salmonella* were found. This survey follows on from a survey carried out during 2001-02 by Welsh Local Authorities and the National Public Health Service for Wales that established updated baseline rates for both pathogens in raw, whole chicken available to consumers in Wales. This survey indicated no difference in *Campylobacter* rates between fresh and frozen samples or between samples taken from retailers and local butchers, but there were significant differences in *Salmonella* rates between fresh and frozen samples and between those sampled from retailers and butchers, with frozen chickens and samples taken from retailers having significantly higher rates. However, the difference in *Salmonella* isolation rate between retailers and butchers was found to be due to the differences in the proportions of fresh and frozen chickens sampled from these locations, with a significantly higher number of frozen chickens (with a higher *Salmonella* rate) being sampled from retailers.
A survey of raw, whole chicken on retail sale in Wales was carried out for ten months between March and December 2003. Samples were examined for Campylobacter and Salmonella. This survey followed on from a previous survey that was carried out in 2001 and 2002 that established up to date baseline contamination rates for Salmonella and Campylobacter in raw, whole chicken (6). The objectives of the 2003 survey were to continue the rolling program of monitoring the Campylobacter and Salmonella contamination of raw chicken available to consumers in Wales, establish seasonality trends for Campylobacter rates in fresh retail raw chicken for 2003 and compare the contamination rates in chicken found in 2001-02 with the rates in 2003. Campylobacter and Salmonella are still considered to be significant food-borne human pathogens in the UK, with estimates having been made of 86 deaths caused by Campylobacter and 119 deaths associated with Salmonella in the UK during 2000 (1).

Four food examination laboratories of the National Public Health Service (NPHS) for Wales and twenty-two Local Authorities participated in the survey, in partnership with the UK Food Standards Agency (FSA). Organization of the survey was by members of the Welsh Food Microbiological Forum (WFMF), a collaboration of Welsh local authorities, the NPHS for Wales, the FSA and the Welsh Assembly Government (5). Lead coordination of the survey was carried out by the NPHS for Wales under the auspices of the WFMF and in partnership with the FSA.
Materials and Methods

Sample preparation. Whole raw chickens (fresh and frozen) were sampled by local authority environmental health departments and submitted to food laboratories for examination. Targets for sampling were 75% fresh and 25% frozen and 70% from retailers (small or large supermarkets selling a variety of produce and usually belonging to a large company) and 30% from local butchers (shops selling predominantly raw meat, usually individually owned and sourcing chickens from local producers). Chickens were stored at <5°C before examination and frozen samples were allowed to defrost. For examination, the neck skin was removed and divided into two equal portions using a sterile scalpel. The carcass was then placed into a sterile bag and manually rinsed for 2 minutes in 225ml of buffered peptone water (BPW), ensuring that all surfaces, internal and external, had contact with the rinse. The rinse was poured into a sterile jar and a portion of neck skin added. Twenty-five ml of this rinse was then pipetted into 225ml of Campylobacter enrichment broth (CEB) and the remaining part of the neck skin added. All media were supplied by Oxoid, Basingstoke, UK.

Campylobacter examination method. The CEB was incubated for 24 hours (+/-6 hours) at 37°C (+/-1°C), followed by incubation at 41.5°C (+/-1°C) for 24 hours (+/-6 hours). The CEB was sub-cultured onto Charcoal Cefoperazone Desoxycholate Agar (CCDA) plates and incubated in a microaerophilic atmosphere (Campygen™, Oxoid, Basingstoke, UK) at 37°C (+/-1°C) for 48 hours (+/-6 hours). All media were supplied by Oxoid, Basingstoke, UK. Presumptive positive colonies were confirmed by oxidase reaction, growth under microaerophilic conditions (Campygen™, Oxoid, Basingstoke, UK) and microscopic determination of cell morphology using carbol
fuchsin stained preparations and examination for typical cells using the oil immersion lens (x400 magnification).

**Salmonella examination method.** The BPW was incubated for 18-24 hours at 37°C (+/- 1°C), followed by selective enrichment of 0.1ml in 10ml of Rappaport-Vassiliadis (RV) and of 1ml in 9ml of Selenite Cystine broth (SC). The RV broth was incubated at 42°C (+/- 1°C) for 18-24 hours and the SC broth was incubated at 37°C (+/- 1°C) for 18-24 hours. The broths were then subcultured onto Brilliant Green agar and Xylose Lysine Desoxycholate agar and incubated at 37°C (+/- 1°C) for 18-24 hours. All media were supplied by Oxoid, Basingstoke, UK. Presumptive positive colonies (non-lactose fermenting with suitable colony morphology) were confirmed using serological (Polyvalent O and Polyvalent H antigens, Murex Biotech, Dartford, U.K.) and biochemical tests (API 20E, Biomerieux, Marcy L’Etoile, France).

**Chi-square (χ²) hypothesis test.** The chi-square test for percentage positive rates was calculated using the Statcalc function of the Epi Info software (2). The α significance level was set at 0.05.
Results and Discussion

Overall, 736 samples were examined, with a weekly mean of 17.5 (range 9-26) and a monthly mean of 74 (range 50-89). The overall positive rates were 73.1% for *Campylobacter* and 5.7% for *Salmonella*. In comparison, the overall positive rates from the 2001-02 survey were 70.8% positive for *Campylobacter* and 8.4% for *Salmonella* from 739 samples taken over 14 months using the same sampling and examination protocols. The *Campylobacter* rates for the two surveys are not significantly different (p=0.320) but the *Salmonella* rates are (p=0.044) with the 2001-02 survey having a higher rate. These *Campylobacter* rates are comparable to other UK studies (4), but the *Salmonella* rate for 2003 is lower than another Welsh study (3). A more detailed breakdown of results for 2003 is shown in Table 1 and a comparison of seasonality in *Campylobacter* rates from fresh samples taken in 2002 and 2003 is shown in Figure 1.

For samples differentiated on the basis of place purchased (retailers or butchers), the results are shown in Table 2. The proportion of retailers and butchers sampled from was 76.8% and 23.2% respectively, with a target of 70% and 30%. There was no significant difference in *Campylobacter* rates in samples taken from retailers and butchers (p=0.555). There was a significant difference between *Salmonella* rates in samples taken from retailers and butchers (p=0.011).

For fresh and frozen samples, the results are shown in Table 1. The proportion of fresh and frozen samples taken was 73.9% and 26.1% respectively, with a target of 75% and 25%. There was no significant difference in *Campylobacter* rates between fresh and frozen samples (p=0.657). There was a significant difference in *Salmonella* rates between fresh and frozen samples (p=0.01).
However, before any further conclusions could be drawn from the differences found in the *Salmonella* rates, a further level of analysis was carried out. The proportion of fresh and frozen samples were analysed by location sampled (Table 3). It was found that, as expected, there was a significant difference in the proportion of fresh or frozen samples taken between the two locations (p<0.001) with significantly more frozen chickens being sampled from retailers than from butchers. This subsequently skewed the statistical analysis of the location-sampled data because the frozen chickens had a higher *Salmonella* rate than the fresh (Table 1). It was therefore concluded that the difference between retailers and butchers in terms of *Salmonella* isolation rate was due to the significant difference in type sampled, rather than a real difference between the two premises types. The reason for the difference between fresh and frozen samples in terms of *Salmonella* isolation rates will require more investigation, as this observation was not made during the 2002 survey when there were no significant differences between either fresh/frozen or retailers/butchers for either *Salmonella* and *Campylobacter* (6).

There also appeared to be a difference between the packaging types of fresh and frozen samples. The majority of frozen samples were packed into bags, whilst the majority of fresh samples were packed in plastic trays covered with plastic cling-film. Of the frozen samples, 22 out of 192 (11%) were assessed as having damaged packaging at the point of sampling. From this total of 22, 14 were positive for *Campylobacter* and 2 were positive for *Salmonella*. Of the fresh samples, 12 out of the 544 (2.2%) were assessed as having damaged packaging at the point of sampling. Of this total, four were positive for *Campylobacter* and one was positive for *Salmonella*. The difference in percentage damaged between fresh and frozen was
statistically significant ($\chi^2=27.57, p<0.005$). The difference in fresh and frozen rates of damaged packaging is probably attributable to the hypothesis that frozen wings, legs or other protrusions may pierce the packaging more easily than for fresh chickens. However, any damage to raw chicken packaging gives the potential for cross contamination to other foods or surfaces, especially when the relative Salmonella rates are considered (Table 1).

The results from this survey provide a measurement of the contamination rates of Campylobacter and Salmonella in raw, retail chicken for the second year in a row and form an important resource for the evaluation of intervention measures carried out during production and slaughter. The results indicate that in relative terms, Campylobacter is still the greater problem within raw chicken, confirming that the current public health interest in the genus is justified. However, the prevalence of Salmonella at a rate of nearly 6% cannot be disregarded.
Acknowledgements

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References


Figure 1 Comparison of *Campylobacter* isolation rates from fresh chicken from 2002 and 2003. Black-data from 2002; grey-data from 2003.
Table 1  Number of *Campylobacter* and/or *Salmonella* positive samples for all, fresh and frozen samples

<table>
<thead>
<tr>
<th></th>
<th>Number <em>Campylobacter</em> positive (%)</th>
<th>Number <em>Salmonella</em> positive (%)</th>
<th>Number of both <em>Campylobacter</em> and <em>Salmonella</em> positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh (n=544)</td>
<td>400 (73.5)</td>
<td>24(4.4)</td>
<td>20 (3.7)</td>
</tr>
<tr>
<td>Frozen (n=192)</td>
<td>138 (71.9)</td>
<td>18 (9.4)</td>
<td>17 (8.8)</td>
</tr>
<tr>
<td>Total (n=736)</td>
<td>538 (73.1)</td>
<td>42 (5.7)</td>
<td>37 (5.0)</td>
</tr>
</tbody>
</table>

Comparison of positive rates for fresh and frozen samples:
*Campylobacter* p=0.657  
*Salmonella* p=0.01
Table 2  Positive rates for *Campylobacter* and *Salmonella* in samples taken from retailers and butchers

<table>
<thead>
<tr>
<th>Sampled from</th>
<th><em>Campylobacter</em> positive rate (%)</th>
<th><em>Salmonella</em> positive rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retailer (n=565)</td>
<td>73.6</td>
<td>6.9</td>
</tr>
<tr>
<td>Butcher (n=171)</td>
<td>71.3</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Comparison of positive rates of samples from retailers and butchers

*Campylobacter* p=0.555
*Salmonella* p=0.011
Table 3 Proportion of fresh and frozen samples analysed by location sampled

<table>
<thead>
<tr>
<th>Location</th>
<th>% Fresh (n)</th>
<th>% Frozen (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butchers</td>
<td>29.4 (160/544)</td>
<td>5.7 (11/192)</td>
</tr>
<tr>
<td>Retailers</td>
<td>70.6 (384/544)</td>
<td>94.3 (181/192)</td>
</tr>
</tbody>
</table>

Comparison of fresh and frozen samples by location sampled $p<0.01$